J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 49(3): 852-858, 2025
Doi: 10.33483/jfpau.1629137

ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ENDEMIC *HAPLOPHYLLUM MYRTIFOLIUM* BOISS.

ENDEMİK HAPLOPHYLLUM MYRTIFOLIUM BOİSS.'UN ANTİMİKROBİYAL VE ANTİOKSİDAN AKTİVİTESİ

Nagehan SALTAN[1], Fatmanur TUNÇ[1]*, Zeynep GÜLCAN[1], Pervin SOYER[2], Yavuz Bülent KÖSE[1]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 26470, Eskişehir, Türkiye
[2]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 26470, Eskişehir, Türkiye

ABSTRACT

Objective: *Haplophyllum myrtifolium Boiss. an endemic species for Türkiye, belongs to the Rutaceae family. In this study, antioxidant and antimicrobial effects of n-hexane and methanol extracts of the aerial parts plant were examined.*
Material and Method: *The total phenolic content of the extracts was examined by a spectrophotometric method using Folin-Ciocalteu reagent (FCR). The DPPH free radical scavenging and ABTS test were applied to determine the antioxidant effect. The antibacterial effects of H. myrtifolium extracts were assayed against Staphylococcus aureus ATCC 29213, Bacillus subtilis NRRL B478* and *Enterococcus faecalis ATCC 51299 by the broth microdilution method.*
Result and Discussion: *The total phenolic content was found to be 44.69±0.021 mg GAE/g for the methanol extract. The methanol extract of H. myrtifolium exhibited higher antioxidant activity (IC_{50} 0.120±0.0008 mg/ml) compared to the n-hexane extract (IC_{50} 0.256±0.015 mg/ml). ABTS test results, the data closest to the TEAC standard was seen in methanol extract (1.783±0.0062 mM). The minimum bactericidal concentrations of the H. myrtifolium methanol extracts varied between 0.125-2 mg/ml and the n-hexane extracts showed antimicrobial activity with the 3 mg/ml concentration.*
Keywords: *Antimicrobial activity, antioxidant activity, Haplophyllum myrtifolium, total phenolic content*

ÖZ

Amaç: *Haplophyllum myrtifolium Boiss. Türkiye'ye özgü bir tür olup, Rutaceae familyasına aittir. Bu çalışmada, toprak üstü kısımlarının metanol ve n-hekzan ekstrelerinin antimikrobiyal ve antioksidan etkileri araştırılmıştır.*
Gereç ve Yöntem: *Toplam fenol içeriği, gallik asit eşdeğeri (GAE) olarak Folin-Ciocalteu reaktifi (FCR) kullanılarak belirlenmiştir. Antioksidan etkiyi belirlemek için DPPH ve ABTS testi uygulanmıştır. H. myrtifolium ekstrelerinin antibakteriyel etkileri Stapylococcus aureus ATCC 29213, Bacillus subtilis NRRL B478 ve Enterococcus faecalis ATCC 51299'a karşı broth mikrodilüsyon yöntemiyle test edilmiştir.*
Sonuç ve Tartışma: *Metanol ekstresi için toplam fenolik içerik 44.69±0.021 mg GAE/g olarak bulundu. H. myrtifolium metanol ekstresi, n-hekzan ekstresine (IC_{50} 0.256±0.015 mg/ml) kıyasla*

* **Corresponding Author / Sorumlu Yazar:** Fatmanur Tunç
e-mail / e-posta: *fatmanurt@anadolu.edu.tr*, **Phone / Tel. :** +90222330580-3702

Submitted / Gönderilme : 29.01.2025
Accepted / Kabul : 16.05.2025
Published / Yayınlanma : 19.09.2025

daha yüksek antioksidan aktivite (IC_{50} 0.120±0.0008 mg/ml) gösterdi. ABTS test sonuçlarına göre TEAC standardına en yakın veri metanol ekstrelerinde (1.783±0.0062 mM) görüldü. H. myrtifolium metanol ekstrelerinin minimum bakterisidal konsantrasyonları 0.125-2 mg/ml arasında değişirken, n-hekzan ekstreleri 3 mg/ml konsantrasyonda antimikrobiyal aktivite göstermiştir.
Anahtar Kelimeler: *Antimikrobiyal aktivite, antioksidan aktivite, Haplophyllum myrtifolium, toplam fenolik içerik*

INTRODUCTION

The *Rutaceae* family is defined worldwide by 319 genera and 6659 species. It is distributed across Africa, the Arabian Peninsula, the Tropical Indian subcontinent of Asia, and Eastern Europe [1]. In Türkiye, the family is represented by 25 species and 4 genera: *Citrus* L., *Haplophyllum* A. Juss, *Dictamnus* L., and *Ruta* L. [2].

Haplophyllum genus was mentioned in the book Flora of Turkey and East Aegean Islands with a total of 19 taxa including 14 species, 3 varieties, and 2 subspecies, 8 of which are endemic [3]. In the book Vascular plants, Adil Güler mentioned 9 species, 6 of which are endemic [4]. Tugay and Ulukuş (2017) introduced *H. sahinii* Tugay & Ulukuş, and Ulukuş and Tugay (2018) introduced *H. ermenekense* Ulukuş & Tugay endemic species [5-6]. The genus with a high rate of endemism is an important gene center for Türkiye. It is popularly known as "Sedo" [2].

Townsend, 1967, who examined the plants of this genus botanically, defined it as a difficult genus in their book Flora of Turkey and, as many species have characteristic facies that are difficult to express in words. They are semi-shrub-like perennial plants with woody lower part and herbaceous upper part. It has 5 petals that range from white to yellow. It has a bracteate cymose flower state. There are 10 stamens with hairy filaments. It has capsule-shaped fruits. It is a plant that grows in sandy, stony and rocky areas at altitudes of 750-2150 m [7].

The genus, which has a wide distribution throughout the world, is widespread distributed from China to Spain, from the North Caucasus to Iran, Iraq, and Somalia. It has traditional use in various diseases in Oman, Sudan, Algeria, Iraq, Saudi Arabia, Egypt, Iran, Türkiye and Mongolia. It has been reported that plants in the *Haplophyllum* genus are used in a wide multiplicity of diseases such as malaria, diarrhea, fever, headache, arthritis, GIS disorders, anemia, skin disorders, gynecological disorders, diabetes, otitis, dermal wounds, allergic rhinitis, asthma [8-16]. According to the evaluation results, whole plants, leaves, fruiting or flowering parts of the plants were used as decoction, infusion, juice, and paste [10,12-14,16]. Additionally, ethnobotanical information on *Haplophyllum* species is crucial for future studies, and these plants are commonly used in traditional medicine in numerous countries.

According to the results of the phytochemical study, *Haplophyllum* species contain essential oils, alkaloids, lignans, coumarins, flavonoids, and terpenes as secondary metabolites [8-12,17-21]. The essential oil has been obtained by various extraction methods using herbs, flowers, leaves, and fruits of plants. According to the analysis results, the main compound classes were classified as terpenic compounds [8-10,20-22].

In a study, it was showed that except the water extract of *H. myrtifolium*, other extracts had anticholinesterase, antityrosinase, antiglucosidase and antiamylase activities [23]. The antitrichomonal effect of *H. myrtifolium* was investigated against *T. vaginalis trophozoite;* its ethanol extract and skimmianine were found effective against it [24]. Due to the chemical content, some *Haplophyllum* species such as *H. patavinum* L., *H. tuberculatum* Juss., *H. hispanicum* Spach, *H. suaveolens* and *H. buxbaumii* are used as antimicrobial, antimalarial, insecticidal in folk medicine [11,16,25,26].

Since many *Haplophyllum* species have important pharmacological properties, their use in traditional medicine has a long history in many countries. The antioxidant and antimicrobial activities of *Haplophyllum* species have been investigated by many researchers using different methods. However, studies on *H. myrtifolium*, an endemic species, are limited. It is crucial to assess its antibacterial and antioxidant activity based on knowledge on its internal and exterior applications related to its ethnobotanical features. Additionally, it is anticipated that the findings about *H. myrtifolium* will serve as the foundation for further pharmacological analyses. In this study, the antimicrobial and antioxidant effect of the endemic *H. myrtifolium* plant, considered as a potential natural resource, were searched.

MATERIAL AND METHOD

Plant Material

H. myrtifolium was collected from Seyitgazi, Eskişehir on May 30, 2022. Plant samples are kept in the Herbarium of Anadolu University Faculty of Pharmacy (ESSE 16362).

Preparation of Extracts

The aerial parts of the plant weighed 10 grams each. 100 ml of hexane (yield 10.1%) and methanol (yield 20.9%) were added and macerated at 150 rpm for 24 hours. This process was repeated three times. It was evaporated with an evaporator at 40ºC. The extracts were kept in the refrigerator at +4ºC.

Determination Total Phenolic Content

The total phenolic content of extracts was measured as gallic acid equivalent by Folin-Ciocalteu method [27].

Determination of DPPH Scavenging Effect

The method developed by Kumarasamy et al. was used for the determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging effect [28]. Gen5 Data Analysis program (BioTek) was used for statistical evaluations. Results are given as mean ± standard deviation. For radical scavenging activity, 50% inhibition concentration value (IC_{50}) was estimated and the formula was used for the % inhibition value.

$IC_{50} = [(A_0-A_1) /A_0)] \times 100$

IC_{50}: 50% inhibitory concentration, A_0: control absorbance, A_1: sample absorbance

Determination of $ABTS^{\bullet+}$ Scavenging Effect

The experiment was performed using the standard Trolox equivalent antioxidant capacity method (TEAC) [29]. This method involves scavenging the $ABTS^{\bullet+}$ (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation) and its comparison with Trolox.

Antimicrobial Activity

The antimicrobial broth microdilution method was employed to assess the Minimum Bactericidal Concentration (MBC) of extracts against standard bacteria species. According to the Clinical and Laboratory Standards Institute (CLSI) in 1999, the determination of MBCs involved identifying the lowest doses necessary to kill 99.9% of the bacterial inoculum [30].

The plant extracts were dissolved in DMSO and prepared by using a two-fold serial dilution technique in a 96-well microplate. The concentrations ranged from 4000 µg/ml to 1.95 µg/ml. Microorganisms were standardized against the McFarland 0.5 reference (1×10^8 cfu/ml) and then bacterial suspensions were diluted 1:100 [31]. Subsequently, firstly 100 µl of each microorganism culture and extracts were added to the plates, which incubated at 37°C for 24 hours. The microorganisms used in the experiment are given in Table 1. Chloramphenicol was used as the standard control, and MHB (Mueller Hinton Broth) medium was used for sterility control. After incubation, 20 µl of 0.01% resazurin dye solution was added to each well. This staining step was performed to differentiate between live and dead cells, with the color change from blue (or green) to pink indicating cell viability. The experiment was conducted in triplicate and the resulting data were later subjected to averaging.

Table 1. Standard test microorganisms

Bacteria	*Staphylococcus aureus* ATCC 29213
	Bacillus subtilis NRRL B478
	Enterococcus faecalis ATCC 51299

Statistical Analysis

The average ± SD of three replicates for every sample was used to present the results. The DPPH techniques' IC_{50} values were determined by the use of linear regression analysis. Test findings for ABTS are reported in millimolar (mM).

RESULT AND DISCUSSION

The antioxidant capacity of two different extracts of *H. myrtifolium* obtained by DPPH and ABTS methods and total phenolic substance content are given in Table 2. The extracts showed differences in their total phenol content and antioxidant capacity depending on the solvent polarity. The effective dose for both extracts was observed to be 2.5 mg/ml. The methanol extract of *H. myrtifolium* showed higher antioxidant activity (IC_{50} 0.1207±0.0086 mg/ml) compared to the *n*-hexane extract (IC_{50} 0.256±0.0150 mg/ml). According to ABTS test results, the data closest to the TEAC standard was seen in methanol extract (1.783±0.0062 mM). Total phenol content was measured using gallic acid equivalents (GAE). The total phenolic content was 44.69±0.021 mg GAE/g for the methanol extract. On the other hand, the *n*-hexane extract contained 37.59±0.007 mg GAE/g of total phenols, indicating a lower content compared to the methanol extract. These results confirm a correlation between the total flavonoid content and antioxidant activity.

Table 2. Antioxidant activity and total phenolic contents of extracts

Extracts and standart	Total phenolic content-mg GAE/g extract	DPPH Test-IC_{50} (mg/ml)	ABTS Test-TEAC (mM)
Methanol	44.69±0.0210	0.1207±0.0086	1.783±0.0062
***n*-Hexane**	37.59±0.0072	0.2560±0.0150	1.512±0.0096
Gallic acid (GA)	-	0.0016±0.0001	2.93± 0.03

In the study conducted by Zengin et al., total phenol content, DPPH and ABTS tests were applied to the extracts of *H. myrtifolium*. Total phenolic content was found in for methanol (34.53 mg GAE/g extract), and petroleum ether (32.32 mg GAE/g extract) extracts. DPPH radical scavenging activity (mg TE/g extract) was listed as follows: methanol 84.49±2.59> water 82.42±4.53> ethyl acetate 67.45± 2.94> petroleum ether 43.77±0.83. The ABTS radical scavenging effect was found for methanol 169.77 mg TE/g extract, and petroleum ether 129.30±1.95 mg TE/g extract [23]. Total phenol content of methanol extract of *H. myrtifolium* (HM-1 and HM-2, Konya) collected from two different localities was calculated as 48.0±0.4 mg GAE/g extract for HM-1 and 56.1±0.8 mg GAE/g extract for HM-2. % inhibition values of ABTS activity were measured as 76.5% for HM1 and 71.1% for HM-2. % inhibition of DPPH activity was observed to be more than 80% for HM-1 and HM-2 [32]. It is observed that the antioxidant effect and total phenol content of extracts prepared with various solvents from plants collected in different localities vary.

Antimicrobial Activity

The MBC values for plant extracts are listed in Table 3. The MBC values of the *H. myrtifolium* methanol extracts varied between 125-2000 µg/ml and the *n*-hexane extracts showed antimicrobial activity only on *E. feacalis* with the 3000 µg/ml concentration and 750 µg/ml on *S. aureus*. According to the MBC values *n*-hexane extracts of *H. myrtifolium* exhibited poor antimicrobial activity with high concentrations. As a result, only the *S. aureus* is more sensitive than other bacteria species.

Table 3. MBC values of *Haplophyllum myrtifolium* extracts (μg/ml)

Extracts and standards	*E. feacalis*	*S. aureus*	*B. subtilis*
Methanol	2000	125	250
***n*-Hexane**	3000	750	-
Chloramphenicol	125	0.97	15.6

-: No activity

Studies on the *Rutaceae* family suggest that this family exhibits antileishmanial effects due to its alkaloid and terpenic compound content [33]. For this reason, when this effect of the endemic *H. myrtifolium* extracts and two isolated alkaloid compounds (Skimmianine and Gamma-fagarine) were investigated, it was reported that they exhibited more than 50% inhibition against promastigotes of *Leishmania tropica* at a concentration of 25 μg/ml [34]. The fact that it showed a significant effect in terms of biological activity led us to plan new studies to investigate this plant further.

In the study conducted by Ashfaq et al., when the antimicrobial activity of various extracts obtained from endemic *H. gilesii* was investigated using the agar well diffusion method, methanol extract showed significant antibacterial activity especially against *S. aureus* and *E. faecalis* [35]. The limited number of studies conducted on *H. myrtifolium*, an endemic plant, reveals the originality of our study.

In the study conducted by Al-Saeghi et al., when the antimicrobial activity of the methanol extract and its subfractions of *H. tuberculatum* was tested against various strains using the agar disk method, it was observed that the activity was similar between different solvents and showed moderate inhibition against the applied strains [36]. In our study, although the methanol extract was more active than the *n*-hexane extract, the methanol extract exhibited low antibacterial activity. It is thought that this difference may have changed due to the different preparation methods and methods used. For this reason, there is a need for a more comprehensive evaluation of the antimicrobial studies on this plant.

Conclusion

Numerous pharmacological actions were present in *Haplophyllum* species, including acetylcholinesterase inhibitory, cytotoxic, cardiovascular, insecticidal, antiprotozoal, molluscicidal, antioxidant, and antimicrobial properties [37]. This study was interested in the antioxidant and antimicrobial effect of *H. myrtifolium* to find new natural sources. It contributed to the literature due to the limited antimicrobial studies on *H. myrtifolium*. This study provided valuable scientific data to the literature and showed that methanol extracts of *H. myrtifolium* may have antibacterial properties. The antioxidant study findings show a parallel relationship between the total phenolic content and antioxidant activity of *H. myrtifolium* extracts. Detailed and comparative studies on this species are planned for future studies.

AUTHOR CONTRIBUTIONS

Concept: N.S., F.T., Z.G., P.S., Y.B.K.; Design: F.T., Y.B.K.; Control: N.S., F.T., Z.G., P.S., Y.B.K.; Sources: N.S., F.T., Z.G., P.S.; Materials: N.S., F.T., Z.G., P.S., Y.B.K.; Data Collection and/or Processing: F.T.; Analysis and/or Interpretation: N.S., F.T., Z.G., P.S.; Literature Review: F.T.; Manuscript Writing: F.T.; Critical Review: N.S., F.T., Z.G., P.S., Y.B.K.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. World Flora Online Web site. (2025). From https://www.worldfloraonline.org/search?query=rutaceae&limit=24&start=0&facet=taxon.family_ss%3ARutaceae&sort=. Accessed date: 28.01.2025.
2. Bizimbitkiler Web Site. (2013). From https://bizimbitkiler.org.tr/v2/hiyerarsi.php?f=Rutaceae. Accessed date: 04.08.2025.
3. Davis, P.H., Mill, R.R., Kit, T. (1988). Flora of Turkey and the East Aegean Islands (Volume 10), Edinburgh University Press, Edinburgh, p.496.
4. Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M.T. (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler), Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, p.835.
5. Tugay, O., Ulukuş, D. (2017). *Haplophyllum sahinii* (Rutaceae), a new species from Central Anatolia (Turkey). Phytotaxa, 297(3), 265-272. [CrossRef]
6. Ulukuş, D., Tugay, O. (2018). *Haplophyllum ermenekense* (Rutaceae), a new species from Turkey. PhytoKeys, 111, 119-131. [CrossRef]
7. Townsend, C.C. (1967). *Haplophyllum* A. Juss. In: P.H. Davis (Ed.), Flora of Turkey and the East Aegean Islands, (pp.496-506). Edinburgh: Edinburgh University Press.
8. Ulubelen, A., Öztürk, M. (2008). Alkaloids, coumarins and lignans from *Haplophyllum* species. Records of Natural Products, 2(3), 54-69.
9. Al-Snafi, A.E. (2018). Pharmacological importance of *Haplophyllum* species grown in Iraq-A review. IOSR Journal of Pharmacy, 8(5), 54-62.
10. Mohammadhosseini, M., Venditti, A., Frezza, C., Serafini, M., Bianco, A., Mahdavi, B. (2021). The genus *Haplophyllum* Juss.: Phytochemistry and bioactivities-A review. Molecules, 26(15), 4664. [CrossRef]
11. Tekin, M., Eruygur, N. (2016). The structural studies on the medicinal plant *Haplophyllum telephioides*. Revista Brasileira de Farmacognosia, 26(5), 544-552. [CrossRef]
12. Hadjadj, S., Bayoussef, Z., El Hadj-Khelil, A.O., Beggat, H., Bouhafs, Z., Boukaka, Y., Tey, M. (2015). Ethnobotanical study and phytochemical screening of six medicinal plants used in traditional medicine in the Northeastern Sahara of Algeria (area of Ouargla). Journal of Medicinal Plants Research, 9(41), 1049-1059. [CrossRef]
13. Batanouny, K.H., Abou Tabl, E., Shabana, M., Soliman, F. (1999). Wild Medicinal Plants in Egypt (Volume 154), The Palm Press, Cairo, pp.1-207.
14. Ghorbani, A. (2005). Studies on pharmaceutical ethnobotany in the region of Turkmen Sahra, north of Iran:(Part 1): General results. Journal of Ethnopharmacology, 102(1), 58-68. [CrossRef]
15. Mossa, J.S., Al-Yahya, M.A., Al-Meshal, I.A. (1987). Medicinal Plants of Saudi Arabia (Volume 1), King Saud University, Libraries Publications Riyadh, KSA, p.340.
16. Al-Yahya, M.A., Al-Rehaily, A.J., Ahmad, M.S., Al-Said, M.S., El-Feraly, F.S., Hufford, C.D. (1992). New alkaloids from *Haplophyllum tuberculatum*. Journal of Natural Products, 55(7), 899-903. [CrossRef]
17. Raissi, A., Arbabi, M., Roustakhiz, J., Hosseini, M. (2016). *Haplophyllum tuberculatum*: An overview. Journal of Herbmed Pharmacology, 5(4), 125-130.
18. Akhmedzhanova, V.I., Angenot, L., Shakirov, R.S. (2010). Alkaloids from *Haplophyllum leptomerum*. Chemistry of Natural Compounds, 46(3), 502-503. [CrossRef]
19. Evcim, U., Gōzler, B., Freyer, A.J., Shamma, M. (1986). Haplomyrtin and (−)-haplomyrfolin: Two lignans from *Haplophyllum myrtifolium*. Phytochemistry, 25(8), 1949-1951. [CrossRef]
20. Saglam, H., Gozler, T., Kivcak, B., Demirci, B., Baser, K.H.C. (2001). Composition of the essential oil of *Haplophyllum myrtifolium*. Chemistry of Natural Compounds, 37(5), 439-441. [CrossRef]
21. Saglam, H., Gozler, T., Kivcak, B., Demirci, B., Baser, K.H.C. (2001). Volatile compounds from *Haplophyllum myrtifolium*. Chemistry of Natural Compounds, 37(5), 442-444. [CrossRef]
22. Hamdi, A., Viane, J., Mahjoub, M.A., Majouli, K., Gad, M.H.H., Kharbach, M., Vander Heyden, Y. (2018). Polyphenolic contents, antioxidant activities and UPLC–ESI–MS analysis of *Haplophyllum tuberculatum* A. Juss leaves extracts. International Journal of Biological Macromolecules, 106, 1071-1079. [CrossRef]
23. Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., Ceylan, O. (2014). A comprehensive study on phytochemical characterization of *Haplophyllum myrtifolium* Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer, skin diseases and type II diabetes. Industrial Crops and Products, 53, 244-251. [CrossRef]
24. Gokmen, A.A., Can, H., Kayalar, H., Pektaş, B., Kaya, S. (2019). *In vitro* anti-*Trichomonas vaginalis* activity of *Haplophyllum myrtifolium*. The Journal of Infection in Developing Countries, 13(03), 240-244. [CrossRef]

25. Çetin, A., Erdoğan, N., Genç, H. (2012). Burdur gölü çevresinin tıbbi ve aromatik bitkilerine bir bakış. Tıbbi ve Aromatik Bitkiler Sempozyumu, 82-190, 13-15 September, Tokat, Turkiye.
26. Massmanian, A., Prieto, J.M. (1996). Dermatitis de contacto por *Haplophyllum hispanicum*, 2ª parte, perfil de una planta agresiva y curativa a la vez. 36 Reunıon del GEIDC, Grupo Espanol de Dermatitis de Contacto, 4 May, Sevilla, Spain.
27. Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 299, 152-178. [CrossRef]
28. Kumarasamy, Y., Byres, M., Cox, P.J., Jaspars, M., Nahar, L., Sarker, S.D. (2007). Screening seeds of some Scottish plants for free radical scavenging activity. Phytotherapy Research, 21(7), 615-621. [CrossRef]
29. Re, R., Pellegrini, N., Proteggente, A., Pannalaa, A., Yanga, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine, 26(9-10), 1231-1237. [CrossRef]
30. Barry, A.L. (1999). Methods for determining bactericidal activity of antimicrobial agents: Approved guideline, national committee for clinical laboratory standards publications, USA, pp.1-32.
31. Humphries, R., Bobenchik, A.M., Hindler, J.A., Schuetz, A.N. (2021). Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. Journal of Clinical Microbiology, 59(12), 10-1128. [CrossRef]
32. Yaman, C. (2023). Phytochemicals and antioxidant activities in methanol extracts of endemic *Haplophyllum* species from Türkiye. Sakarya University Journal of Science, 27(3), 687-696. [CrossRef]
33. Plock, A., Sokolowska-Köhler, W., Presber, W. (2001). Application of flow cytometry and microscopical methods to characterize the effect of herbal drugs on *Leishmania* sp. Experimental Parasitology, 97(3), 141-153. [CrossRef]
34. Ostan, I., Saglam, H., Limoncu, M.E., Ertabaklar, H., Toz, S.O., Ozbel, Y., Ozbilgin, A. (2007). *In vitro* and *in vivo* activities of *Haplophyllum myrtifolium* against *Leishmania tropica*. The New Microbiologica, 30(4), 439-445.
35. Ashfaq, S., Hussain, M., Bibi, N., Alam, J., Junaid, M., Rehman, S.U. (2019). Antimicrobial and cytotoxic potential of *Haplophyllum gilesii* (Hemsl.) CC from Northern Pakistan. Pakistan Journal of Agricultural Research, 33(1), 146-153. [CrossRef]
36. Al-Saeghi, S.S., Hossain, M.A., Al-Touby, S.S.J. (2022). Characterization of antioxidant and antibacterial compounds from aerial parts of *Haplophyllum tuberculatum*. Journal of Bioresources and Bioproducts, 7(1), 52-62. [CrossRef]
37. Mitra, J.R., Eira, B.N., Bhawmick, A.K., Remon, M.K.I., Enan, M.E., Muhit, M.A., Zohora, F.T. (2022). Phytochemical and pharmacological aspects of genus *Haplophyllum (Rutaceae)*: A review. International Journal of Health Sciences, 6(S9), 4454-4485.